Intraspecific and interspecific variation in workers of the *Formica rufa* group (Hymenoptera: Formicidae) in Sweden

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The variation in the number of erect hairs on the different parts of the body of large workers of the taxonomically difficult *Formica rufa* group (excluding *pratensis-nigricans*) was analysed. Samples of about five individuals from 122 more or less randomly chosen nests from different parts of Sweden were compared by a principal component analysis of a matrix of correlation coefficients. The four clusters obtained by this analysis (corresponding to the species: *polyctena, rufa, aquilonia,* and *lugubris*) vary considerably in the characters studied. Despite the large variation good discriminating characters were found permitting the specific identification of single large workers in most cases. The four species do not vary geographically as is shown by the lack of correlation among the geographical and morphological distances between the nests.

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Introduction

The *Formica rufa* group, the mound-building wood ants of central and north Eurasia, consists of a number of morphologically and ecologically very similar species. There has been much confusion as to the classification of this group and the revisionary studies by Yarrow (1955) and Betrem (1960) have greatly helped to clarify the situation. It is now generally accepted that there are five or six species in the *rufa* group: *rufa* L., *polyctena* Först., *aquilonia* Yarrow, *lugubris* Zett., *pratensis* Retz., and *nigricans* Em. There is disagreement about the taxonomic status of *pratensis* and *nigricans*. Some authors consider them to be specifically distinct (e.g. Collingwood 1979) while others treat them as one species (*pratensis,* eg. Kutter 1977). When trying to identify specimens of the *rufa* group using modern quides (Collingwood 1979, Dlussky & Pisarski 1971, Kutter 1977) it becomes apparent that the taxonomy of this group is not sufficiently understood. There seems to be no problem in separating the *pratensis-nigricans* complex from the other species, at least when queens are available, whereas problems in identifying *rufa, polyctena, aquilonia,* and *lugubris* often arise. These problems obviously originate in an insufficient knowledge of the intraspecific variation in this complex.

The great similarities between the species in the *rufa* group have been demonstrated by numerous studies. Differences have been found in the number of erect hairs on different parts of the body especially in the workers (Betrem 1960, Collingwood 1979, Dlussky & Pisarski 1971, Kutter 1977, Yarrow 1955). Microsculpture on head and gaster also varies between species (Collingwood 1979, Yarrow 1955) but preliminary scanning electron microscopy (SEM) studies by Pamilo et al. (1979) and by myself yielded no clear differences between species. Male genitalia do not show species-specific characteristics (Dlussky & Pisarski 1971), nor did fluorescent pigments (Gösswald & Schmidt 1959a, b), haemolymph proteins (Loos-Frank 1978, Schmidt & Hess 1973), or chromosome number (n=52) (Hauschteck-Jungen & Jungen 1976). Allozyme studies by Pamilo et al. (1979) demonstrated exceptionally low genic variation between the species of the *rufa* group. They also

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showed that this group is clearly separated from the other *Formica* species (perhaps excluding *F. truncorum* F. which is sometimes included in the *rufa* group despite its distinctive morphology (Yarrow 1955)). Bergström & Löfqvist (1973) identified 46 volatile substances in Dufour's gland in workers of *rufa, polyctena,* and *pratensis-nigricans*. *F. rufa* and *polyctena* were almost identical while *pratensis-nigricans* showed a slightly different pattern. Bühring et al. (1976) investigated volatile substances from different body parts of *rufa* and *polyctena* workers and found that these species differed in one substance—nonanal—that occurred in *rufa* only. However, very little is known about intraspecific variation (in non-morphological characters almost nothing) and therefore the small differences found might be insignificant.

Thus, although species of the *rufa* group have been compared in many different aspects no clear differences between the species have been found except between *pratensis-nigricans* and the other four species. As a matter of fact it is far from clear that there are five or six species. It might be just two—*rufa* and *pratensis*—or even more than six species (Collingwood 1979). To bring this problem closer to a solution requires a better understanding of the relations between intraspecific and interspecific variation in this group. This goal can best be achieved by studying morphological characters from a large and unbiased sample from as large an area as possible. Morphological characters are easily accessible compared to other characters that might be useful at a later stage to improve the classification based on morphology.

There are two major taxonomic problems in the *rufa* group—those concerning the *pratensis-nigricans* complex and the *rufa-polyctena-aquilonia-lugubris* complex. The latter is dealt with in the present study.

Characters

I have restricted this investigation to an analysis of the numbers of erect hairs (those that are more or less raised above the body surface) in workers from Sweden. This choice was made for the following reasons. Workers are easier to collect than queens and males and I did not succeed in getting enough sexuals for this study. Placement and abundance of erect hairs on the body and the appendages of workers probably offer the best means for the classification of this group (see references above). Other attributes of the workers that vary between nests and hence could be used for classificatory purposes are the microsculpture of the frontal area of the head and the first tergite of the gaster and also the size and intensity of dark areas on the head and promesonotum. According to my own preliminary SEM studies microsculpture seems to vary less than Collingwood (1979) has indicated and moreover I find it difficult to quantify the differences. The latter is also true for the dark areas. Erect hairs can occur almost everywhere on the worker ant and my first idea was to count them on all body parts. Regarding the legs the number of erect hairs on the femora and tibiae were counted in 15 samples (five individuals/sample) including all the four supposed species and *pratensis-nigricans*. It turned out that the hind femur discriminated far better between the samples than did any of the other parts which is shown by F in Tab. 1 and that the hind femur was highly correlated with the other parts (Tab. 2). This means that counts of hairs on the legs can be restricted to the hind femur with only a small loss of information and the saving of a lot of work.

In a previous study (Douwes 1979) on part of the present material, erect hairs were counted on the head (excluding antennae, mouthparts, and eyes), alitrunk (excluding the anteriormost tip of pronotum which is sometimes hidden by the head, and propleura), hind femur, margin of petiole scale (the hairs seen in profile), and the second gastral tergite. Thus some parts were excluded either because they were considered to be insignificant or because it was impossible to count the number of hairs with certainty (eyes, propleura and most of gaster). The areas of the head and alitrunk distinguished in counting the hairs are shown in Douwes (1979 Fig. 3). Due to symmetry only one half (when possible the left one) was studied. The analysis based on these 11 characters yielded four distinct groups (= species) (Douwes 1979). Since the characters are highly correlated it is possible to reduce the number of characters without loss of information. Thus ignoring the clypeus, upperside of head, metapleuron-metanotum (area 7 in Fig. 3 in Douwes 1979), and second gastral tergite did

Table 1. Analysis of variance of numbers of erect hairs on legs of *rufa* group workers. 15 samples (5 indiv./sample).

Source of variation	d.f.	Frontleg		Midleg		Hindleg	
		femur	tibia	femur	tibia	femur	tibia
variance between nests	14	12.406	3.104	21.384	5.620	21.499	9.693
variance within nests	60	0.542	0.283	0.851	0.444	0.618	0.671
F (between/within)		22.894	10.983	25.127	12.656	34.777	14.445

Table 2. Correlation (erect hairs) between hind femur and other leg parts in the 75 individuals in Tab. 1.

	Correlation coeff.				
	Hind	Mid		Front	
	tibia	femur	tibia	femur	tibia
Hind femur	0.91	0.97	0.95	0.93	0.89

not change the estimates of morphological distance between the samples as was shown by the high correlation coefficient (0.992) between two matrices of euclidean distances (Sneath & Sokal 1973), one matrix based on all the 11, the other on the reduced number of characters.

In the present investigation the remaining seven characters and the number of hairs on the eye were analysed (Tab. 5).

Samples

This investigation is restricted to Sweden although this country covers only a small fraction of the total distribution area of the *rufa* group. Yet I think that the results based on Swedish material might be of general interest because all the four supposed species are widely distributed in Sweden (Collingwood 1979) and hence much of the variation in this group should be present here.

Samples were taken from 122 nests each sample usually consisting of five large workers (alitrunk size > 2.16 mm). More individuals (usually ten) were studied from some nests because the workers appeared to be of an intermediate character. The reason for studying large workers was that there is a relationship between body size and abundance of hairs ranging from no correlation in the least hairy species (*polyctena*) to a strong positive correlation in the most hairy species (*lugubris*) (Douwes 1979). Thus the species differ more in large workers than in small ones.

The samples were collected in the following way. Areas (= localities) 0.5–2.0 ha in size were surveyed and from all the nests that were found a sample was taken. Sometimes colonies of interconnected nests were found and in such cases a sample was taken from one nest only. In central and north Sweden nests of the *rufa* group were very common in some localities and it was impossible to collect from all of them. The sampling localities are shown in Fig. 1. It is obvious from this map and from what has been said above that the samples are far from random from a geographical point of view. For instance, southernmost Sweden is strongly over-represented but this bias will not seriously affect the results. However, it should be emphasized that the samples were not selected with respect to the appearance of the ants. Thus I did not attempt to get typical samples of the four species nor intermediates between them. I just collected *rufa* group samples, sorted out *pratensis-nigricans* using Collingwood (1979) and Kutter (1977), and analysed the rest.

Numerical analysis

The basic unit in this study is the sample and the first (and main) task is to find out if these samples can be grouped on the basis of their similarity in the eight characters mentioned above and to evaluate the taxonomic significance of such groups. Nests of the *rufa* group are supposed to be composed of one single species; at least there is no evidence of mixed nests (except for two *rufa*-group species from North America, King & Sallee 1951) and my results show "pure" samples (nests).

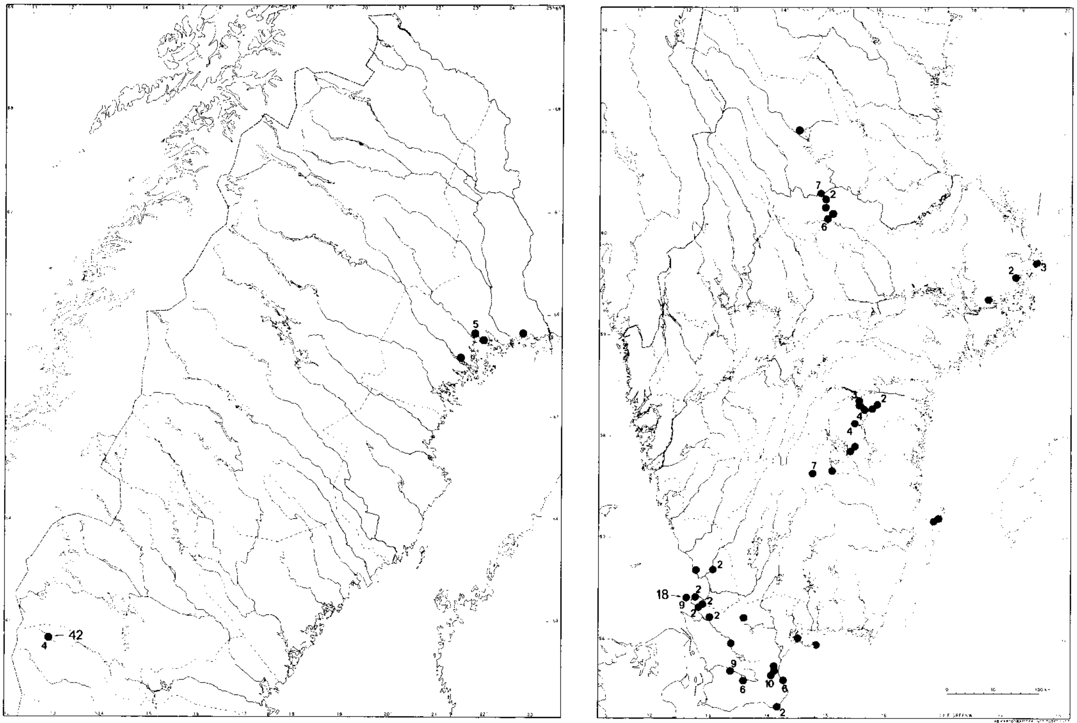

Fig. 1. Sampling sites. Each dot represents one or in a few cases two localities (see text). The localities mentioned in the text are marked by large figures. The number of samples when more than one per dot is given by the small figures.

The methods used here to estimate similarity and analyse taxonomic structure are described in Sneath & Sokal (1973). The computation were done by the MINT system (F. J. Rohlf) on a UNIVAC 1100/80 at the Lund University Computing Centre.

The relationship between the samples in all eight characters have to be summarised to a comprehensible pattern in a few (maximally three) dimensions. Diffferent techniques could be used to reduce the dimensions of the variation but I believe that in this particular study an ordination method is the most appropriate one, since almost all the variation can be shown in two dimensions. Thus the principal components were extracted from a matrix of correlation coefficients between characters and the distribution of the samples on the two first principal axes was examined. If the samples have a clumped distribution this may represent different species. It is obvious from Fig. 1 that the samples are geographically grouped and hence the clusters in the principal component analysis might show geographical variation within a species rather than different species. In order to solve this problem the geographical variation within the clusters was analysed by correlating a matrix of euclidean distances (expressing morphological distance) with a matrix of geographical distances between the nests from which the samples were taken.

The estimate of similarity is based on eight characters all of them being numbers of hairs. These are more numerous on some parts of the body than on others which means that the similarity coefficient is more influenced by some characters than by others. One group of characters—number of hairs on the legs—has been undervalued so far, since only hairs on the hind femur were counted. To compensate for this I have used the hind femur value twice. Thus the characters have different weights and this can be

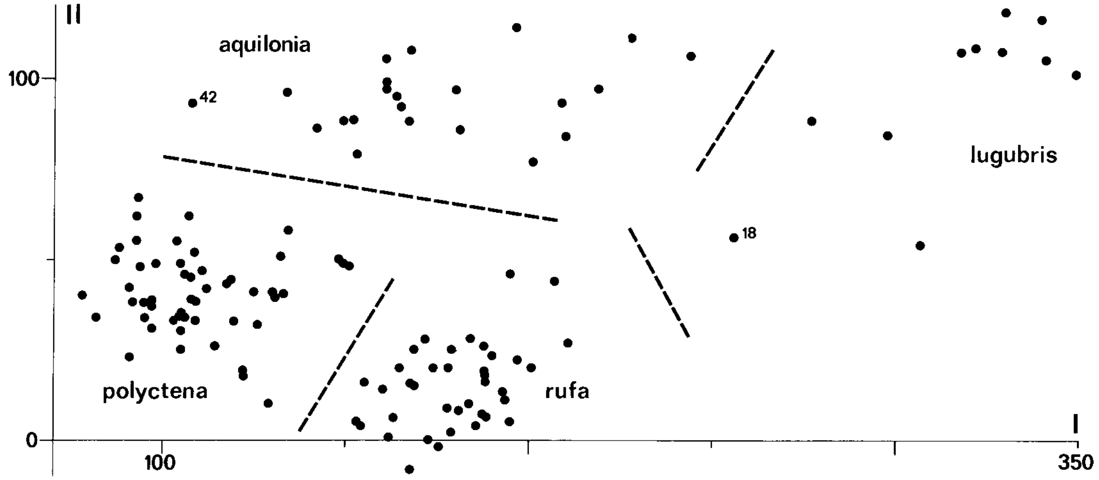

Fig. 2. Principal component analysis. The position of the 122 samples on the first two principal axes. The first two eigenvalues represent 89 % of the variance. The clusters have been interpreted as different species—the boundaries between them being shown by broken lines. The locality number is given for some of the samples.

improved when the intrasample variation is considered. The weighting of a character is inversely proportional to the intrasample variance of that character. However, because the variance (within samples) increases with the mean, the mean intrasample variance can not be correctly estimated unless the data are transformed. I have used a $\sqrt{}$-transformation of the crude data which made the variance (standard deviation) independent of the mean. Thus the sample mean of square root number of hairs was divided by the mean intrasample standard deviation and this new value was used to estimate the similarity.

Having found that the samples represent different species the next step in this investigation is to present a picture of the intraspecific variation, i.e. the variation within and between nests. A multivariate estimation of the overall variation would provide the information desired. However, the assumptions for a multivariate analysis of variance are not satisfied here and therefore I have separately calculated the correlations among the characters and the variation in each character. The standard deviations representing the different levels of variation (within samples, between sample means, and total of samples) were estimated. In order to avoid a bias in the estimates of the total standard deviation only five individuals (the first five in my lists) were considered in samples containing six or more individuals.

In the analyses of intraspecific variation (excluding geographical variation mentioned in connection with the principal component analysis) I have used the crude data, since there is no reason to use transformed data for these kinds of analyses and the crude data show in a simpler way the characters discriminating between the species.

Results

In the principal component analysis 89 % of the variance was removed by the first two principal axes which means that the relationships between the 122 samples is very well represented in Fig. 2. Four groups can be discerned although the exact delimination of the groups is difficult. Thus, so far it can be suggested that there are four species in this complex, especially since the morphological characters of the four groups agree with those of *rufa, polyctena, aquilonia,* and *lugubris* according to Collingwood (1979). An alternative explanation would be that the clusters reflect the geographical variation within a species—this variation being discontinuous due to the geographically separated areas from

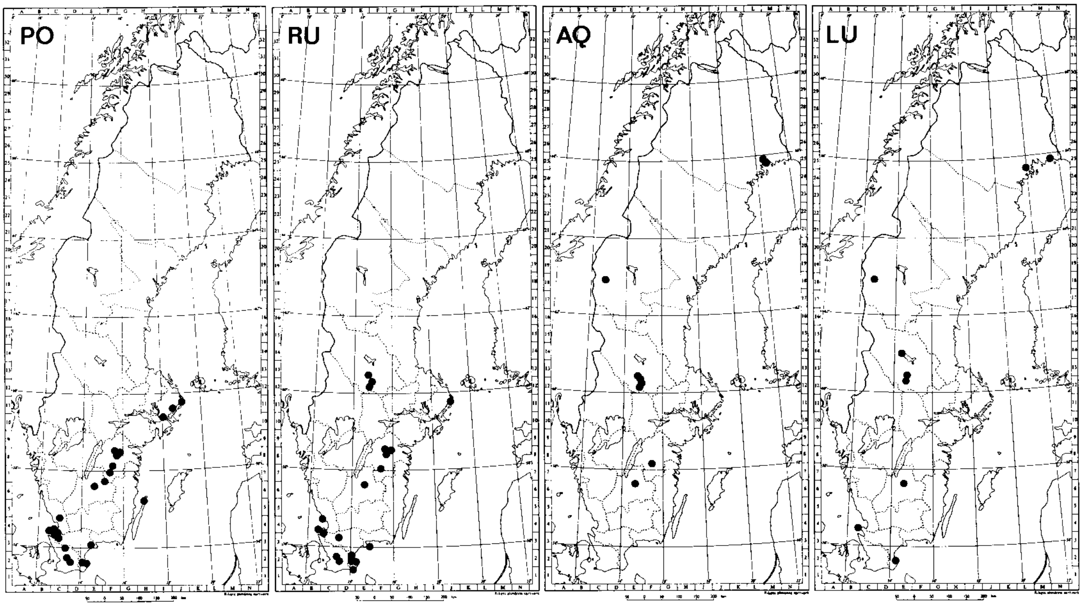

Fig. 3. Distribution of the samples of PO (= *polyctena*), RU (= *rufa*), AQ (= *aquilonia*), and LU (= *lugubris*).

which the samples were collected. However, these areas have nothing to do with the clusters obtained from the principal component analysis, since all clusters are widely distributed (Fig. 3) and, moreover, there is no correlation between morphological and geographical distance within the clusters (Fig. 4). Thus it can be safely concluded that the four clusters represent different species.

F. polyctena and *rufa* form compact clusters that are separated by a distinct, though narrow gap. The delimination of these two species from *aquilonia* and *lugubris* seems to be more problematic. *F. rufa* is clearly separated from *aquilonia* as is also the latter species from *lugubris*. *F. polyctena* and *aquilonia* overlap in one sample (42 in Fig. 2) that cannot be assigned to either species on morphological grounds alone. Considering the geographical position of locality 42 which is 800 m above sea-level and far outside the range of *polyctena* (Figs. 1, 3) this sample belongs undoubtedly to *aquilonia*. The most problematic sample is the one between *rufa*, *aquilonia* and *lugubris* (18 in Fig. 2). It has been classified as *lugubris*, since it seems to be slightly closer to that species than to any of the others. Unfortunately the rather few samples of *lugubris* provide only a rough picture of the variation in that species.

No geographical variation was found in these species (Fig. 4). The location of the nests from which the samples were taken is denoted by 1×1 km squares on the Swedish National Grid System. Since in almost all cases a locality (0.5–2 ha) is included in such a 1×1 km square and different localities are from different squares, the bottom line in Fig. 4 (geographical distance = 0) shows the morphological distances between samples from the same locality. Comparing the morphological distances at different geographical distances in Fig. 4 it becomes apparent that there is less variation among nests near to each other than in the species as a whole (in Sweden) at least in *polyctena*, *aquilonia*, and *lugubris*.

The lack of geographical variation in these species is somewhat unexpected, since it has been claimed that the degree of similarity between species varies geographically (Pamilo et al. 1979) indicating an intraspecific geographical variation. Possibly Sweden is too small an area to demonstrate geographical variation in these species.

In Tab. 5 the intra- and internest variation is shown by the last two columns. As could be

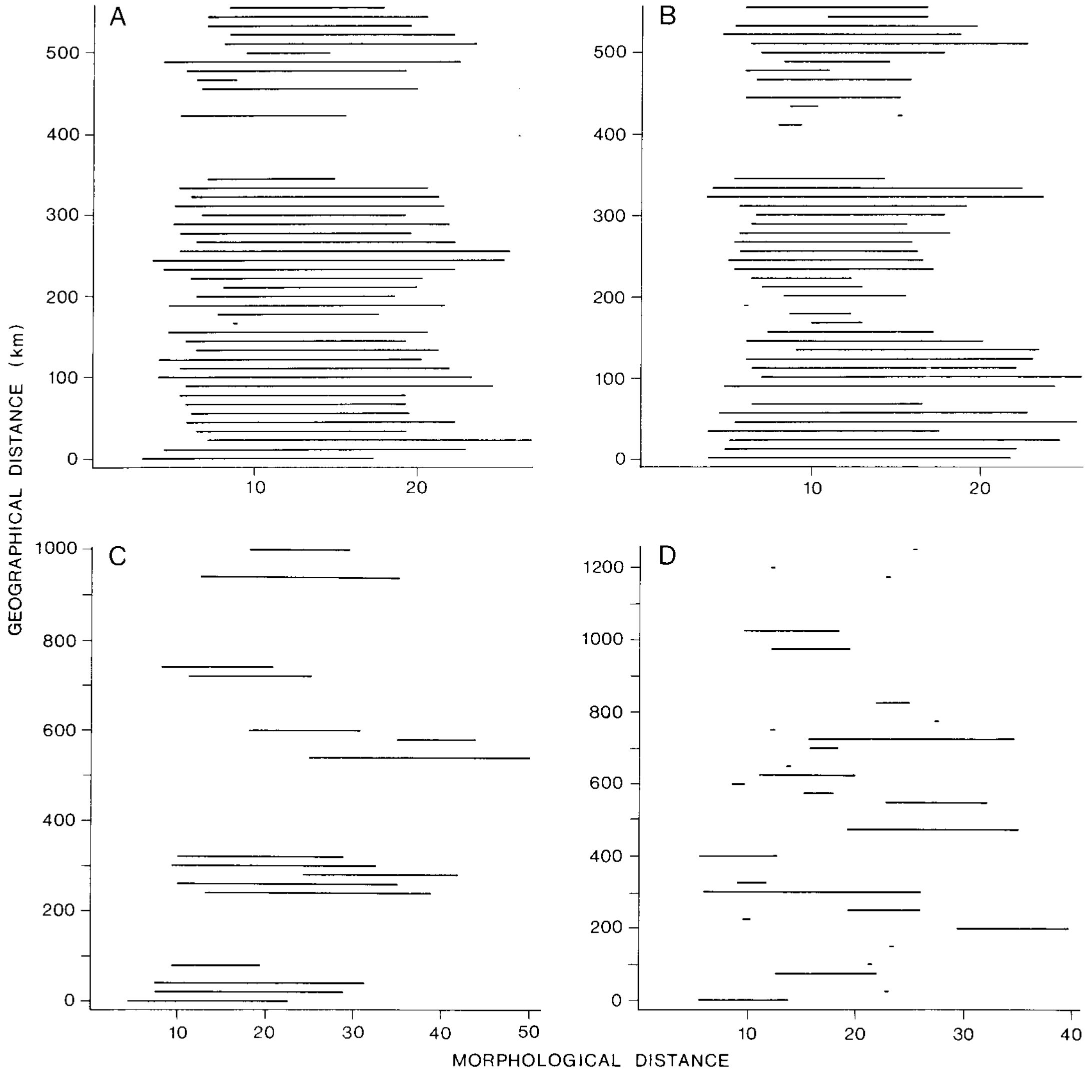

Fig. 4. Geographical distance plotted against the morphological distance (euclidean distance) between the samples of the four species. — A = *polyctena* (correl. coeff. = 0.04). — B = *rufa* (–0.05). — C = *aquilonia* (0.12). — D = *lugubris* (0.14). The horizontal lines connect the extremes of the euclidean distance coefficients (morphological distance). The bottom line shows the range of morphological distances between samples from the same locality (geographical distance = 0).

expected the main component of variation is between nests. Due to their haplo-diploid system, workers from the same nest are very closely related, especially when there is only one queen, and there should be almost no genetic variation within a nest. It is also shown that the standard deviations are highest in *lugubris* and lowest in *polyctena*. However, the standard deviation is positively correlated with the mean and when the effect of the size of the mean is removed the four species show the same degree of variation (Fig. 5). There might be a difference between *rufa-polyctena* on the one hand and *aquilonia-lugubris* on the other; the latter being more variable between nests as compared to within-nest variation.

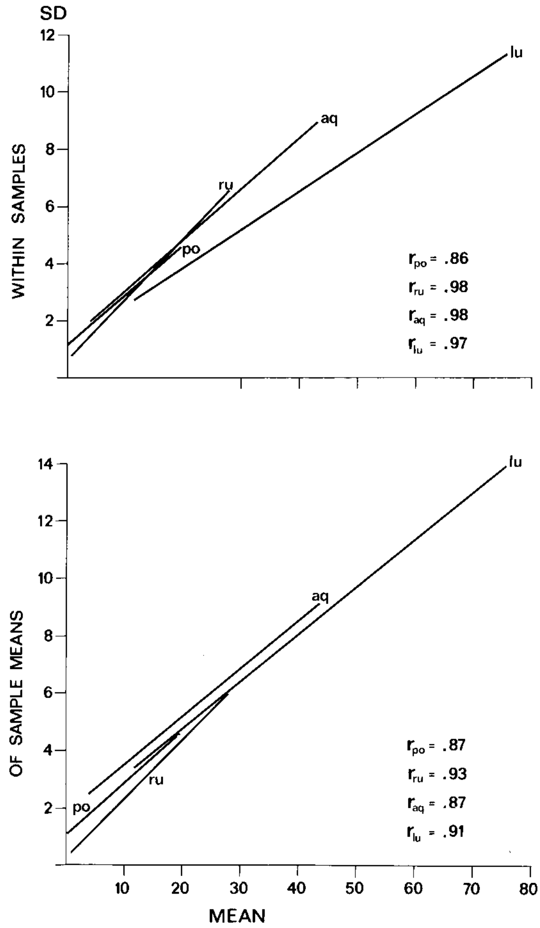

Fig. 5. Regression of standard deviation (SD) on the mean in the eight characters. — po = *polyctena*. — ru = *rufa*. — aq. = *aquilonia*. — lu = *lugubris*. — r = correlation coefficient.

Table 3. Correlation (of sample means) between number of erect hairs on: uh = underside of head, bh = back of head, eye, pn = pronotum, mn = mesonotum, pp = propodeum, pe = petiolus and fe = hind femur. *F. polyctena* is shown above the diagonal and *rufa* below. Product-moment correlation coefficients.

	uh	bh	eye	pn	mn	pp	pe	fe
uh	—	0.44	0.17	0.70	0.24	0.39	0.36	0.35
bh	0.47	—	0.13	0.53	0.08	0.11	0.24	0.49
eye	0.16	0.51	—	0.20	–0.12	0.06	0.17	0.35
pn	0.08	–0.18	–0.29	—	0.17	0.54	0.45	0.49
mn	0.31	0.29	0.05	0.39	—	0.39	0.34	0.10
pp	0.22	0.00	–0.24	0.61	0.47	—	0.67	0.37
pe	–0.16	–0.10	0.00	0.40	0.22	0.37	—	0.47
fe	0.00	0.24	–0.05	0.21	0.19	0.47	0.44	—

Table 4. Correlation (of sample means) between number of erect hairs on different parts of the body and the hind femur (see Tab. 3) in *aquilonia* (above the diagonal) and *lugubris* (below). Product-moment correlation coefficients.

	uh	bh	eye	pn	mn	pp	pe	fe
uh	—	0.74	–0.17	0.58	0.40	0.58	0.54	0.47
bh	0.72	—	0.02	0.64	0.57	0.77	0.68	0.72
eye	0.66	0.56	—	0.06	0.00	0.15	0.34	0.42
pn	0.62	0.57	0.17	—	0.61	0.92	0.77	0.63
mn	0.02	0.09	–0.33	0.58	—	0.69	0.59	0.65
pp	0.39	0.65	0.20	0.62	0.45	—	0.85	0.76
pe	0.40	0.65	0.17	0.64	0.40	0.93	—	0.70
fe	0.52	0.74	0.42	0.58	0.07	0.56	0.53	—

The separating characters between the species are found by inspecting the second and third columns of Tab. 5 and comparing the figures shown there with the correlation coefficients among the characters in Tabs. 3 and 4. Before going into detail, some remarks on the results presented in Tab. 5 should be given. *F. polyctena* is shown to have more hairs on all body parts than acknowledged by previous authors (see references above). Collingwood (1979) states: 'Some samples of *F. rufa* tend to approach the hairless condition in *F. polyctena*....'. My results indicate that the opposite is true, i.e. that *polyctena* tends to approach the hairy condition in *rufa*. The presence or absense of erect hairs on the back of the head has invariably been used to separate *rufa*+*polyctena* and *aquilonia*+*lugubris*. As much as 39 % of the *rufa* workers in this study had one or more erect hairs on one half of the back of the head and also a few *polyctena* workers had one hair (though difficult to see). Nevertheless, the number of erect hairs on the back of the head when correctly expressed are very useful for identification. Size and number of hairs on the eyes are also used to separate *rufa*+*polyctena* and *aquilonia*+*lugubris* by Collingwood (1979) and others. There is much overlap in numbers of hairs between all species (Tab. 5) whereas there are differences in the size of the hairs. *F. lugubris* has larger and more erect hairs than *rufa* and *polyctena*. *F. aquilonia* is very variable in this respect.

Table 5. Number of erect hairs on different body parts. Means (x̄) and 95 % intervals (x̄ ± 1.96 SD). — SD_1 = total standard deviation. — SD_2 = standard deviation of the sample means. — SD_3 = standard deviation within samples (nests). — uh = underside of head. — bh = back of head. — pn = pronotum. — mn = mesonotum. — pp. = propodeum. — pe = petiolus. — fe = hind femur.

character	species	x̄	x̄ ± 1.96SD_1	x̄ ± 1.96SD_3	SD_2	SD_3
1/2 uh	*polyctena*	1.69	0.0–5.1	0.0–4.2	1.23	1.27
	rufa	5.75	2.0–9.5	3.3–8.2	1.59	1.24
	aquilonia	3.74	0.0–8.4	0.6–6.9	2.05	1.60
	lugubris	11.67	4.3–19.0	5.5–17.8	2.06	3.14
1/2 bh	*polyctena*	0.03	0.0–0.2*	0.0–0.2	0.06	0.10
	rufa	0.75	0.0–3.8*	0.0–2.5	0.72	0.89
	aquilonia	6.01	0.0–14.3	0.0–13.6	2.38	3.85
	lugubris	30.46	9.6–51.3	13.2–47.7	5.73	8.80
eye	*polyctena*	5.30	0.0–11.8	0.0–11.0	1.82	2.89
	rufa	5.06	0.0–10.8	0.8–9.3	2.11	2.15
	aquilonia	10.94	4.0–17.9	7.3–14.6	2.90	1.86
	lugubris	12.34	0.6–24.1	2.7–21.9	3.50	4.90
1/2 pn	*polyctena*	3.62	0.0–12.5*	0.0–8.5	2.94	2.49
	rufa	20.24	9.2–31.3	13.4–27.1	4.54	3.49
	aquilonia	5.90	0.0–18.3*	0.0–15.6	3.08	4.94
	lugubris	41.45	23.6–59.3	29.4–53.5	7.00	6.14
1/2 mn	*polyctena*	2.72	0.0–7.9*	0.0–5.9	1.71	1.61
	rufa	10.84	3.3–18.4	5.8–15.9	3.06	2.59
	aquilonia	3.34	0.0–9.3*	0.0–6.9	1.87	1.81
	lugubris	16.49	5.4–27.6	7.3–25.7	4.18	4.71
1/2 pp	*polyctena*	3.35	0.0–9.3*	0.0–7.1	2.07	1.89
	rufa	14.60	6.0–23.2	9.0–20.2	3.42	2.84
	aquilonia	3.95	0.0–11.3*	0.0–10.5	2.15	3.35
	lugubris	29.54	15.5–43.6	19.4–39.7	4.50	5.19
1/2 pe	*polyctena*	4.05	0.0–8.5	1.4–6.7	1.88	1.35
	rufa	8.40	4.0–12.8	5.7–11.1	1.79	1.36
	aquilonia	5.59	0.8–10.4	2.0–9.1	1.86	1.81
	lugubris	14.76	8.8–20.8	10.4–19.1	2.32	2.23
fe	*polyctena*	19.73	7.7–31.7	11.1–28.3	4.39	4.38
	rufa	27.80	12.3–43.3	14.2–41.4	6.02	6.93
	aquilonia	43.36	19.9–66.8	24.9–61.8	8.96	9.42
	lugubris	75.70	39.4–112.0	47.1–104.3	11.30	14.57

* Due to the skewed distribution of the character not estimated from x̄ and SD. The interval shows 95 % of the variation in the specimens studied.

The species are said to differ in the number of hairs on the extensor surface of the hind femur (see references above). I find it difficult to decide which hairs belong to the extensor surface so I have examined all of the femur including the distal end.

When trying to find the characters that most effectively separate the species, the overlap in single characters and the correlation between the characters is of interest. The usual way of describing how to identify species is to give a diagnostic key. I think that in this case it is more illuminating to compare the species two by two. One such species pair—*polyctena* and *lugubris*—does not concern us here since they are sufficiently dissimilar not to be confused with each other and moreover an intermediate between these two species would be indistinguishable from *rufa*.

The second and third columns in Tab. 5 show the overlap in single characters. The left of these two columns is aimed at showing the character state in 95 % of the workers and the right one the character state in 95 % of the samples of five workers/nest from the Swedish population. It should be pointed out that these are large workers (altitrunk length > 2.16 mm) only.

F. polyctena and *rufa* differ in their overall degree of hairiness—the hairs being distributed over the body in approximately the same way in the two species. There is a great deal of overlap in most body parts but least so in the pronotum which, however, has to be used in combination with at least one other character for a certain discrimination between these species. The second best character—the propodeum—is strongly correlated with the pronotum (Tab. 3) and therefore adds less information than does the third best character—the mesonotum—which is much weaker correlated with the pronotum. These two characters in combination are probably sufficient to distinguish between *polyctena* and *rufa* in almost all cases. When necessary the propodeum could also be examined.

F. polyctena and *aquilonia* present serious identification problems. They overlap considerably in all characters but the back of the head and the hind femur and unfortunately these two characters are correlated (Tab. 4). The problem is that *aquilonia* sometimes has strongly reduced number of hairs thereby very much resembling typical *polyctena*. Probably nests of *aquilonia* occur that cannot be separated from *polyctena* by the number of hairs on the back of the head and on the hind femur. Possibly this is the case in southern Finland (Collingwood 1979, Pamilo et al. 1979).

F. rufa and *aquilonia* are fairly easy to tell apart by the number of hairs on the back of the head and on the propodeum. These two characters are positively correlated in *aquilonia* (but not in *rufa*, Tabs. 3, 4) which means that specimens of *aquilonia* that are indistinguishable in the back of the head can be identified by the propodeum, or in critical cases also by the mesonotum. The back of the head separates effectively *rufa* and *lugubris*, as does the pronotum or the propodeum between *aquilonia* and *lugubris*.

Thus provided the abundance of erect hairs is given in absolute terms (which previous authors have not done), the four species can be identified in almost all cases when large workers are available. This is true for at least the Swedish populations.

The assumption made that each nest contains a single species can now be checked by the composition of the 122 samples. Thus no individual that indisputably belongs to an alien species (according to the characters given above) was found in the samples of *polyctena*, *rufa*, and *lugubris*. One *aquilonia* sample (from locality 42) contains two specimens that agree with typical *polyctena*. However, all individuals in that sample are similar to *polyctena*. Consequently no evidence for mixed nests are given by the samples.

The distribution of the samples investigated gives a rough picture of the distribution of the four species in Sweden (Fig. 3). *F. aquilonia* and *lugubris* seem to occur all over the country, the former probably being absent from the extreme south and both being more abundant in central and north Sweden. *F. polyctena* and *rufa* are apparently restricted to southern Sweden. Thus the four species are sympatric in a large area and two or more species frequently occur near to each other in the same habitat (all four species were found together in one locality).

The results presented here are strictly only valid for the Swedish populations and although the situation might not be very different elsewhere in Europe, similar studies in other areas are of great value. It is quite possible that the classification of the *rufa* group is still incomplete (apart from the *pratensis-nigricans* problem) and that a study of more material and other characters will split the clusters obtained here into further species.

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